ABSCISSION IN MIRABILIS JALAPA

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(WITH PLATE XIII AND TWO FIGURES)

The purpose of this investigation

Although the phenomenon of abscission has received no small amount of attention, a study of the literature fails to discover any real uniformity of opinion as to the precise steps involved. This divergence I have already indicated in an earlier paper.[1] As I shall take occasion at a future time to attempt a general critique of the whole subject, my present purpose is a restricted one, namely, the consideration of a more recently published account of abscission in the common "four o'clock" (*Mirabilis jalapa*), by E. HANNIG.[2] My curiosity touching the matter, so far as this plant is concerned, was aroused by HANNIG's statement that the mode of abscission, which is common only to *Mirabilis* and *Oxybaphus*, does not accord with any previously observed accounts, and is made by him, therefore, to represent a new type, while his figures appeared to me not to support his contention. It seemed to me rather that a more exhaustive study of even the published drawings of LOEWI[3] and of TISON[4] alone would have led HANNIG to see a strong resemblance between those and his own figures. Before material of *Mirabilis* was available for my own observation, I ventured therefore to believe that he was not justified[5] in formulating a new type of abscission. Since then I have obtained an abundance of material, and after a careful study of the process in question in both stems and leaves, I find myself unable to alter my opinion.

[1] Abscission. Ottawa Nat. **28**:41–52; 61–75. 1914.

[2] Untersuchungen über das Abstossen von Blüten, etc. Zeitschr. Bot. **5**:417. 1913.

[3] Blattablösung und verwandte Erscheinungen. Proc. Vienna Acad. **1**:166–983. 1907.

[4] Recherches sur la chute des feuilles. Mém. Soc. Linn. Normandie **20**:125, 1900.

[5] LLOYD, *loc. cit.*, p. 72.

The purpose of this paper is to support a challenge of the correctness of HANNIG's views and of the evidence brought to support them, and to substitute evidence to show that abscission in *Mirabilis* does not represent a new type. To do this will entail a detailed account of the process as understood by me, and to compare this with that of HANNIG as exhaustively as possible.

Digest of Hannig's view

HANNIG recognizes in general two methods of abscission of flowers (that is, of the supporting axis), namely, (*a*) by the solution of the middle lamella of the cells of the abscission zone; and (*b*) by the complete dissolution of an entire layer of cells. The latter constitutes the new type in question, and is, according to HANNIG, "of a very peculiar sort." His account runs as follows:

Two or three cell layers of the abscission zone, which is 12–20 cells thick, are destroyed and go completely over into solution. This destruction proceeds first by the thinning of the membranes of the affected cells. These membranes become more strongly refringent, while the cell contents take on a granular character. The process is first recognized by the absence of intercellular spaces, followed by granular degeneration of the cell, ending finally in the liquidation of the whole layer of tissue. The process begins at a particular point under the epidermis, which is soon broken. The tear then extends around the whole cortex and finally inwardly into the pith. The vascular bundles appear to be broken across mechanically. The persistence of starch in the abscission cells, while the surrounding tissues, except the endodermis, lose it, is asserted, but even those cells which are reduced to extremely thin membranes, simply because of their too rapid destruction, also retain it. Starch in the abscission cells is therefore of no particular significance. HANNIG was unable to find any evidence, by means of suitable reagents, of chemical alteration of the cell walls to distinguish them from those of neighboring cells. From these conclusions the further one is drawn that the entire cells of the abscission layer, without recognizable previous alteration of the cell membranes, go into solution (p. 430).

He further holds that there is present in all plants which shed their leaves in laboratory air a preformed primary abscission zone ("Trennungsschicht") which is more or less sharply set off from the neighboring tissues. It is pronounced in some species (*Salvia*, *Fuchsia*, *Impatiens*, etc.), but less so in *Begonia* and *Mirabilis*. This zone is to be seen also at the base of the internode. It is the less marked the older and thicker the internode, but still can be recognized, since the cells are smaller and display new transverse cell walls. In *Impatiens*, however, these new walls are so infrequent that the abscission zone is recognizable thereby with difficulty. The manner of separation is the same in leaf, stem, and flower (pedicel, peduncle).

The larger nodes offering the best opportunities for observation, HANNIG further says that it is not the entire abscission zone which is dissolved, but a layer ("Lösungsschicht") of a few cells which are not distinguishable beforehand in any way. This dissolved layer is placed usually in the middle, but sometimes toward the base of the abscission zone, and without reference to the direction of the cell layers. Contrasting *Mirabilis* with forms like *Impatiens* (in which the cells of the abscission zone are set free by the dissolution of the middle lamella), it is pointed out that, while in these the abscission surfaces exposed after rupture are granular or pulverulent in appearance, due to loosened cells, those in *Mirabilis* are mucilaginous. In this plant then the abscission zone is only a more or less broad band of tissue, in the approximate middle of which the separation layer ("Lösungsschicht"), itself not in any way differentiated as to its cells, occurs. This zone is conceived by HANNIG only as a zone of tissue capable of choristic response, to adopt FITTING'S term.

The present account of abscission in Mirabilis

The preceding account, being a digest of HANNIG'S statement, has been set out with some fulness and detail in order to obviate the possibility of a criticism which may be in any way superficial or gratuitous. HANNIG'S resulting contention that in *Mirabilis* (and in the allied *Oxybaphus*, which I have not examined), a type of abscission obtains which is "quite new and distinct," is

presently examined in the light of the facts as they are now to be presented.

THE ABSCISSION ZONE

While it is known that abscission in the internode occurs, in general, near its base, it is impossible to connect it with visible structural peculiarities, preformed specificially with respect to the process. HANNIG says as much in admitting that, although frequently or usually delimited by the smallness of the cells or the occurrence of transverse divisions, these marks are not always to be discovered.

In very young internodes there is nothing at all to enable one beforehand to fix upon the tier (usually one only) or tiers of cells which become involved later. In older structures the configuration of the cells for a short distance *both above and below* the node is practically identical, and, although transverse walls are plentiful in the prosenchyma, one cannot regard these as having any more definite relation to abscission than the still older walls. Just as abscission sets in one can frequently recognize the evidence of a renewal of cellular activity in a zone of several tiers of cells, for in these may occur many new, very thin transverse walls ("secondary meristem" of earlier authors). Abscission finally intervenes usually at the upper limit of this zone (pl. fig. 4), and not in the middle or below, although several tiers of cells may finally take part. There is no other structural zone which deserves designation in this connection. Since, however, even such a zone as this occurs only sometimes in older organs, in which a general rejuvenescence then appears to be demanded before abscission can supervene, it can hardly enable us to delimit or define a reactive zone. This zone of rejuvenescence is the "Folgermeristem" of VON MOHL, in which his "Trennungsschicht" arises, and, as WIESNER[6] has pointed out, the latter does not always arise in a secondary meristem. KUBART[7] took the trouble to point out the proper use of these terms, and there appears to be no good reason to change their usage.

[6] Über Frostlaubfall, etc. Ber. Deutsch. Bot. Gesells. **23**:49. 1905.

[7] Die organischen Ablösung der Korollen, etc. Sitz. Akad. Wiss. Wien. **115**: 1491. 1906.

Evidence, however, will be furnished to show that a zone of tissue, here designated the abscission zone, composed of a number (in large internodes about 10–12) of tiers of cells *below* the separation layer, but not above, shows enough visible alteration, quite aside from cell divisions and distinguishable by optical and chemical means, to warrant the conclusion that it constitutes a physiologically active zone concerned in abscission. It is not simply a secondary meristem, if by this term is implied a renewal of transverse cell division, since this does not always occur, but a mass of cells going through the initial steps, consisting most obviously of chemical alteration of the cell wall, leading to abscission, and of which only some cells finally conclude the process. These latter, which are sufficiently active to go far toward and many of them finally to conclude separation, constitute the separation layer. A secondary meristem may or may not intervene.

THE CENTERS OF ABSCISSION ACTIVITY AND THE DIRECTION OF ITS PROPAGATION

According to HANNIG, abscission begins under the epidermis (which soon tears) and travels around the cortex, finally passing through the vascular bundles and into the pith.

The fact appears to be that the first step in abscission (separation) in internodes takes place in the innermost cells of the cortex at two points in a plane normal to the plane of the opposed leaves. From these points the process is propagated outwardly, inwardly, and around the stem, but more rapidly toward the center of the pith than toward the epidermis. Indeed, separation is usually completed in the pith before the epidermis is ruptured (pl. figs. 2, 4). Because of the longitudinal growth of the separation cells, there is a considerable displacement (0.1–0.2 mm.) of the parts to be separated, and there is a synchronous rupture of the passive portions of the xylem. We therefore find the vascular tissues to be in an advanced state of disruption (text fig. *2d;* pl. fig. 14) before the epidermis is ready to break. When this finally occurs, the fracture of the separation layer is most easily accomplished.

In young stems and leaves the same procedure seems to be followed, although, because of the delicacy of the organs and the rapidity with which abscission is consummated, it is more difficult to follow. There is undoubtedly, however, a great deal of irregularity. Instances have been seen in which the epidermis was fractured and the wound gaped open, in the manner suggested by HANNIG's fig. 9, before the separation was completed in the pith.

CHEMICAL ALTERATION IN THE WALLS OF THE ABSCISSION CELLS

Proof that separation is preceded by a chemical alteration in the cell wall is the following.

(1) If a section (preferably a fairly thick one) is treated with strong KI/I, the walls of the abscission cells will appear to have an indefinite pale greenish hue. If the reagent is washed away gradually, as the yellow of the iodine disappears, the walls show a pale blue, which fades away as the washing is prolonged. An open diaphragm is necessary. This color reaction[8] is much more pronounced if the section is first boiled for a minute in weak (4 per cent) HCl, which, because of a previous alteration of the cell walls, attacks them but does not affect the remainder. Not merely the cells actually involved in separation, but *all the cells below the separation plane for a distance of 0.5 mm.* (in a large node) show the blue reaction, but in less and less degree the farther from the separation plane. Above this the transition is sudden, only a portion of the adjacent cell walls of the cells immediately above being altered.

(2) In earlier stages of abscission, however, the blue reaction is not visible, but an indication of chemical alteration is to be seen in the failure as compared with the adjoining, unaltered cells, to color strongly with iodine.

(3) The staining capacity of the wall of the separation cells is obviously reduced. They will not hold Bismarck brown (pl. fig. 9), so that they become almost colorless, while the unaltered

[8] The reaction was observed under circumstances of evident physiological significance by GREEN (The soluble ferments and fermentation, p. 97) and by myself (Development and nutrition of the embryo . . . . in the date). Ann. Rep. Mo. Bot. Gard. **21**:103. 1910.

walls remain deeply stained. The relation is the same to ruthenium red, but not so markedly.[9] One must distinguish between the staining capacity of the entire wall of the abscission cell before elongation sets in, and that of the delicate membranes which continue to invest the protoplast during and at the culmination of elongation. The latter seem to stain readily, at least more so than the former.

(4) The optical quality of the walls is clearly altered, since, with the iris diaphragm at a given opening, a much more narrowly outlined object picture is obtained than in the case of an unaltered cell. HANNIG states that this condition obtains (pp. 428–429).

(5) There is a readily appreciable amount of swelling of the cell walls, most marked in those which are thicker. Consequently, the thick-walled cells of the collenchyma and prosenchyma sheath are most favorable for observation. This phase appears to be passed through quite quickly. Transverse sections display this condition most abundantly (text figs. 1, *a*, *b*; 2, *a*, *b*, *c*; pl. figs. 5, 6, 12, 13).

(6) Following this the walls become altered to such an extent that, when successfully stained with ruthenium red, the substance of the wall appears as a flocculated mass, separated from the protoplasm by a delicate membrane (pl. figs. 6, 13). Whether the flocculation is due to the preservation in alcohol or to the action of ruthenium red[10] matters less than the fact that the walls are in a condition either of flocculation or in one which allows it. When in this condition the walls frequently show breaks of such character that they can be explained only on the supposition that it is in the condition of a gel. As abscission nears completion, this granular matter is much reduced in amount, apparently by hydrolysis. The product may be absorbed by the abscission cells, for which it may very well be regarded as a source of energy. It is not superfluous to insist that this granular matter is not cytoplasm, although it may easily be mistaken for it, especially as displacements of the

[9] Successful differential staining is obtained best by means of quite dilute solutions.

[10] I have noticed that ruthenium red flocculates the pectic (?) mucilages derived from ripe fruits of *Diospyros*, but have been unable to see any flocculation in fresh, unstained material of *Mirabilis*.

delicate cell walls confuse the microscopic picture. That the cells whose walls are thus being changed are not degenerating, witness the fact that cell divisions may be taking place, and the condition of the nucleus and cytoplasm.

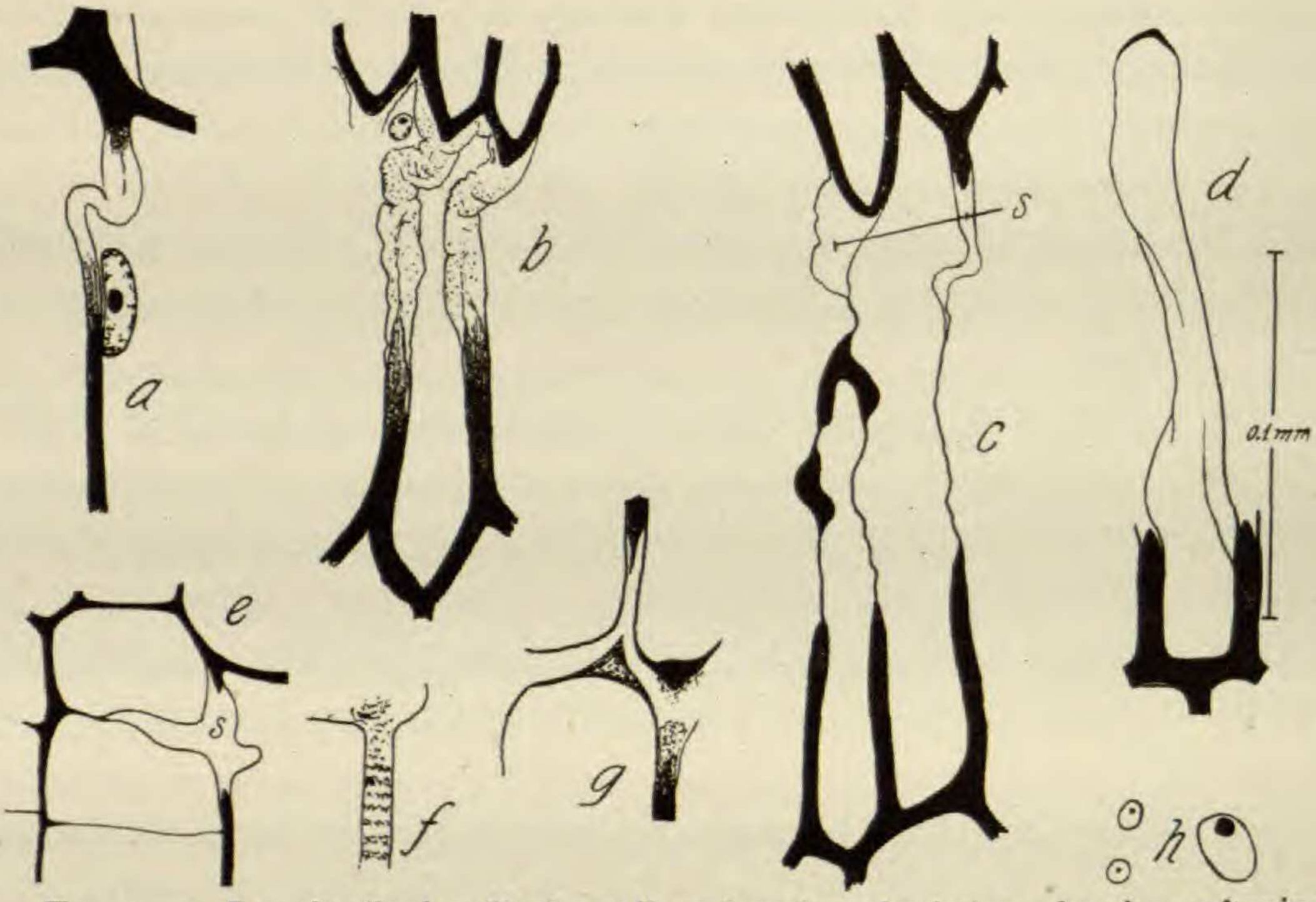

FIG. 1.—*a*, Longitudinal wall of a cell undergoing abscission, showing softening in a restricted zone, near which the nucleus lies (cf. pl. fig. 9); *b*, more advanced condition, in which the walls are very much altered, but remain bounded by a delicate tertiary membrane (cf. pl. figs. 5, 6, 12, 13); *c*, condition of extreme extenuation of the tertiary membranes, seen separated in the upper part of the figure; portion of unaltered wall remains suspended in these membranes (cf. pl. fig. 10, a portion of which was selected for this figure; also pl. fig. 11); *d*, cell after separation, the tertiary membrane intact and evidently disarticulated at its upper end from two more distal cells (cf. pl. fig. 8); *e*, partially separated pith cells, the tertiary membranes remaining intact; *f*, the flocculated remains of a hydrolyzed membrane held between, and broken up by, the extending tertiary membranes in the pith; this condition may be seen in preparations represented by pl. fig. 7; *g*, mutual disarticulation of the ends of adjacent cells; *h*, small nuclei from parenchyma cells near the abscission layer, but not taking part in abscission; and a large one from an abscission cell in an advanced stage; drawn to identical scale.

THE SEPARATION LAYER; ITS COMPOSITION

Only one tier of cells may be involved in the act of separation (pl. figs. 5, 7), this usually in smaller, younger leaves and internodes, except that in young leaves there is a tendency to increase the number of layers involved. In larger nodes, in which there

is considerable thickening of the walls of certain tissues (especially the prosenchyma sheath and cortex), from one to four or five tiers of cells (pl. figs. 3, 4, 15) may undergo some of the changes in form leading to separation, although it is seldom that the cells of more than one tier actually complete the process. This is usually the uppermost, although occasionally one below this may conclude separation, but in a restricted region only. In no case, however, do all the superimposed separation tiers pass entirely across the stem, for in the pith usually one tier only is engaged, only occasionally more. The maximum number of tiers activated is to be

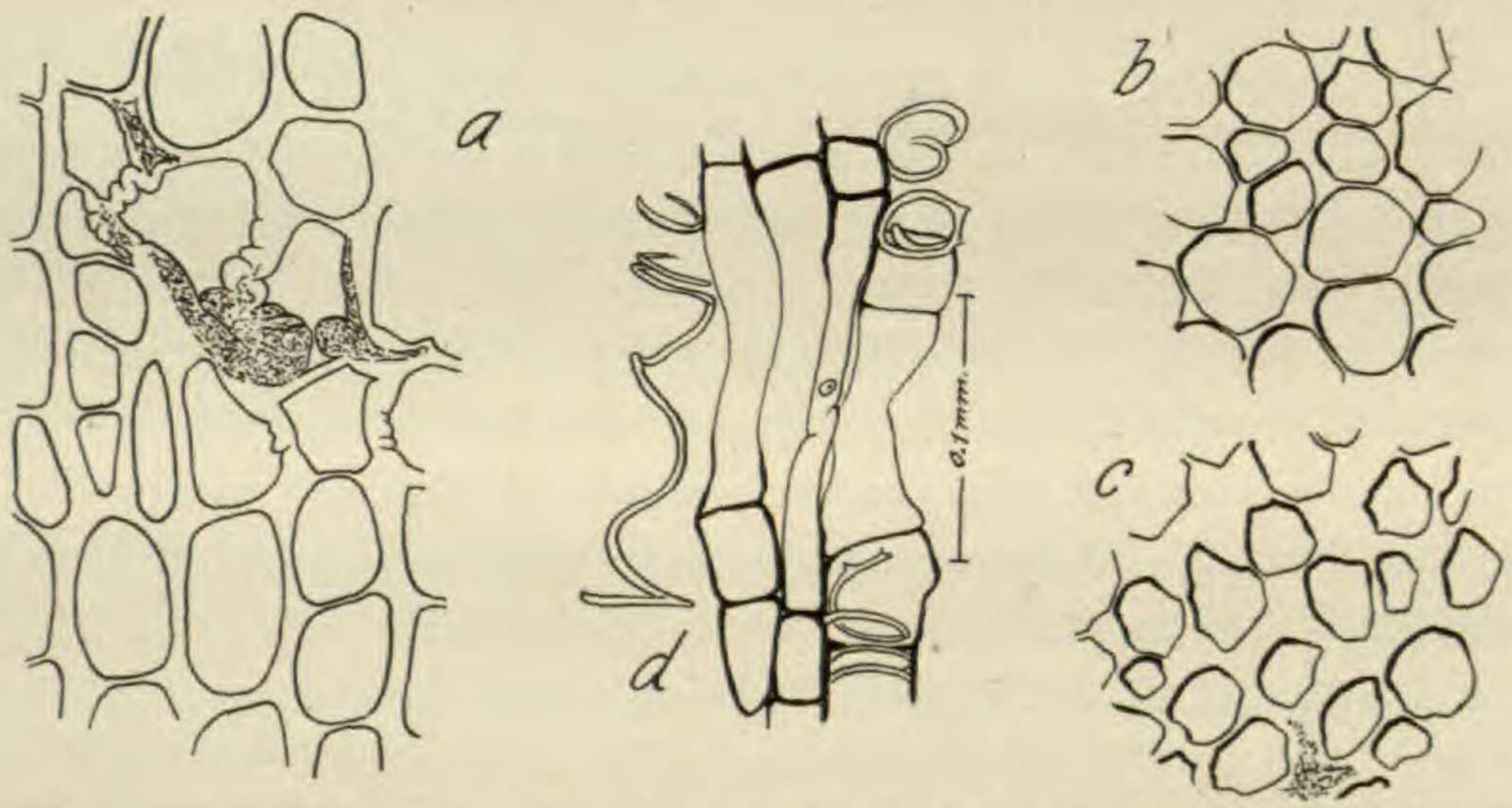

FIG. 2.—*a*, Transverse section through a portion of the prosenchyma sheath, showing swollen walls; *b*, *c*, transverse sections through cortex; in *b* the walls are unaltered, in *c* they are swollen, but still incompletely hydrolyzed; *d*, longitudinal view of a few wood parenchyma cells ready for separation, with adjacent wood vessels fractured (cf. pl. fig. 14).

found in the prosenchyma sheath, where the resistance against longitudinal growth is greatest, due to the thickness of the cell walls and to the presence of the vascular tissues.

The mechanical resistances doubtless affect also the direction taken by the separation layer. Although, broadly speaking, this follows the direction of the tiers of cells, it tends to depart from it, and, as the epidermis is approached, to pass into the cell tiers below (pl. figs. 3, 4), as if the tendency to lie normal to the abscission cell axes was to some extent overcome by the tendency to lie normal to the stem axis. The maximum irregularities in direction

occur where there is the greatest heterogeneity of structure or maximum mechanical resistance, namely, in the oldest stems and in the vascular tissues, so markedly indeed that I have frequently observed in some plants an entire misdirection of the plane of abscission, in that it comes to lie parallel with the vascular bundles for some distance. Conversely, the greatest regularity is seen in the pith and medullary rays, where, however, in spite of the homogeneity of the tissues, minor local departures from the ideal path may be observed (pl. figs. 1–4).

CYTOLOGICAL BEHAVIOR OF ABSCISSION CELLS

a) THE RÔLE OF STARCH.—Previous to abscission, there is an abundance of starch in the general region below the separation layer, while it is withdrawn from above it except from the starch sheath. As abscission progresses, the starch disappears below, from the middle of the pith last. From this point it has disappeared usually by the time abscission is well advanced throughout its whole extent. In the early stages of abscission starch is to be found within all the separation cells themselves, but it largely or entirely disappears as abscission is completed. The place where the absence of starch is first to be noted is in the prosenchyma sheath next to the starch sheath. The latter also loses starch in the vicinity of the abscission tissues. When abscission is complete, I have observed in some cases various but always small amounts of starch, a few large grains in the middle of the pith and a very few scattered grains elsewhere or none. The fact therefore appears to be that, as abscission progresses, the starch in the abscission cells decreases until it is very materially reduced in amount or entirely disappears. The inference is that it is used by them as a source of energy, expended in the active growth leading to separation.

HANNIG described the abscission layer as being marked by a transverse band of starch grains, but adds that those cells which are reduced to extremely thin membranes and are more or less collapsed still contain starch. He suggests that this is due to the rapid autolysis of these cells; but the purpose of this essay is to

show that such autolysis does not occur, and, if this be true, HANNIG'S inference is not justified. The appearance of collapse or compression is accidental, and that the abscission cells in *Mirabilis* should appear so is not to be wondered at, in view of the facts presented in this paper. While some starch grains may indeed persist even until after abscission is completed, the granules are quite obviously very much reduced in size, so that, in a particular plastid, the stroma enveloping the granules may readily be seen between them, and the granules of starch may appear as minute points.

To draw a sure inference from the presence of starch in, or its absence from, separation cells is at best precarious. For example, the separation cells in *Hydrangea*, in which "maceration" occurs as in *Impatiens*, retain their starch; while the immediately neighboring cells lose it. But, when abscission is complete, the separation cells, while entirely loosened from each other, are quite alive. In such a case as this the starch may not be used at all, and may be retained because the loosening of the cells prevents its movement (this being essentially HANNIG'S explanation), or it may have been secreted in amount much in excess of that needed. But it is, in any event, doubtful if the loosening of the cells would prevent the movement of starch.

b) THE BEHAVIOR OF THE CELL WALL.—Evidence has already been brought forward to show that, previous to any changes in the dimensions of the cells involved in abscission, there occurs an alteration of the cell walls. The degree of alteration appears not to be the same throughout the whole of the cell wall, judging by its behavior during those steps preceding separation. For the sake of simplicity, I take an ideal case, that of a fairly thick-walled cell of the prosenchyma sheath, in which the physiological activity proceeds in a plane normal to the axis of the cell (pl. fig. 15), it being premised that this plane may lie in any oblique direction (pl. fig. 10) passing through any of the walls. The changes observable are presented seriatim.

(1) The cell wall is softened in the previously described manner, most completely, however, in a narrow zone nearer the upper

end of the cell and therefore nearer the plane of final separation, in the event that this supervenes (text fig. 1, *a*, *b*).

(2) The cell grows longitudinally, the maximum extension of the walls occurring where the maximum softening has taken place, and concurrently with it. The length of the cell may increase four or fivefold. The total length of the longest cells in the prosenchyma sheath at the time of separation has been found to be 0.2 mm. or slightly more (text fig. 1, *c*, *d*).

The protoplasmic utricle also becomes greatly extenuated during this period of growth and the cytoplasmic membrane is most delicate. The nucleus is disposed in the transverse zone of elongation, usually increases in size, and remains perfectly normal in appearance until the completion of separation and even still later (text fig. 1, *h*). It may lie against the wall or be suspended in or near the axis of the cell. Occasionally a very delicate transverse wall is laid down just previous to or at the time of elongation. Transverse walls may have been formed still earlier, but the fact that the cellular activity may take this form during the process leading to separation must be taken as evidence of vigor rather than of degeneration. Further, it is not possible to suppose that this behavior is consonant with a loss of turgor, supposed by HANNIG to occur, while the disappearance of the starch suggests a constant accession of solutes for maintaining turgor.

FURTHER ANALYSIS OF THE CHANGES WHICH OCCUR IN THE CELL WALL

It is of prime importance, in view of the purpose of this paper, to analyze fully the changes undergone by the walls which display elongation. These are more readily appreciable in the thickest of them. The wall shows first of all evidence of chemical alteration resulting in a physical change which allows it to be drawn out.[11] There is no evidence that the middle lamella alone is altered, but rather does it appear that the whole wall, excepting only a delicate membrane limiting the lumen of the cell, is softened,

[11] For a review of various accounts of the way in which the cellulose membrane is affected by enzymes, see JONES, L. R., Pectinase, etc. N.Y. Agric. Exp. Sta. Techn. Bull. 11. Nov. 1909.

probably by hydrolysis (text fig. 1). This remaining membrane[12] it is which continues, during elongation of the cell, to invest the protoplasm, and which, because of the disappearance of the remainder of the wall, comes into intimate contact with those of neighboring cells (text fig. 1, *c*). Being very soft and delicate membranes, on coming into contact with each other they cling together and appear as a single membrane, which is optically scarcely resolvable as double, so that its composition must be argued from the occasional separation of its components (pl. fig. 11), and from their conjoint greater thickness. These changes are for obvious reasons, more prolonged where the walls, or portions of the walls, are most thickened, as in the collenchyma and prosenchyma sheath, and are therefore more readily seen in such tissues (pl. figs. 5, 6, 12, 13).

Not only are these thin membranes proper to neighboring cells separate from each other, but they become separated also at their upper ends (in some cases at their lower ends) from the thick membrane of the cells in the next tier above, only the lower ends of which are chemically altered, but enough for this. The iodine reaction demonstrates this to be the case. That this separation of walls actually obtains can be proved by taking rather thick sections of suitable material and, after a slight treatment with 5 per cent sodium hydrate, or weak hydrochloric acid,[13] pulling the portions separated by the abscission layer apart on the slide, when the separation cells pull away in many cases without breaking. Investing their free ends one usually finds a thicker portion of wall (pl. fig. 8) evidently derived from the chemically altered but unstretched membranes with which the ends of the abscission cells were in contact (text. fig. 1, *d*, *g*). It is more difficult to

[12] These membranes investing the protoplasts of the abscission cells were seen by TISON. His sketches, however, do not convey an accurate conception of their appearance, nor does he seem to have followed their genesis closely; for example, he recognizes no growth. LOEWI, on the other hand, saw the elongation of the walls, without following the changes in the walls themselves, or comprehending the nature of the thin walls consequent on these changes.

[13] One is not compelled to use these or any reagents, but they facilitate the obtaining of particularly fine preparations. Since the treatment is not sufficient to hydrolyze even the thinnest of the membranes, it cannot be objected to.

demonstrate that these membranes are free by means of untreated sections, although not at all impossible.

The result of all this is to produce a separation cell, the ends of which are invested by thicker, physically relatively unaltered walls (text fig. 1, *d*) with a transverse zone between them, narrow at first, but becoming quite wide at length, of extremely thin membrane. Viewed *en face*, the thicker portions of the wall show their shallow pits, well seen in the prosenchyma sheath, and between them and the thin portions the pits are seen to disappear and the membrane itself to show the granulation or flocculation due to the chemical alteration (pl. fig. 15). If, as has been premised, the thinning out has proceeded in irregular, more or less oblique zones, very various topographic conditions (pl. fig. 10) ensue without any violation of principle.

Although the above view of the nature of the thin walls seems to accord entirely with the facts, it remains true nevertheless that they could be accounted for by supposing that, as the old walls become softened and broken down, entirely new thin walls are laid down by the growing protoplasm. This is TISON's view. While it is altogether possible, or even probable, that some new wall material is being laid down, the optical evidence is against the idea that the wall is entirely new. One can, if with some difficulty, resolve a relatively unaltered membrane in contact with the protoplast in the portions of the cell which are not elongated, and can determine its continuity with the thin membranes in the zone of elongation. The continuity comes out clearly when the chemical alteration of the rest of the wall is far enough advanced so that the optical differences presented by the primary and secondary membranes on the one hand and the tertiary membrane on the other are obvious (pl. figs. 6, 13; text fig. 1, *b*, *f*).

The process as described is the same for all living elements, for example, cambium cells and wood parenchyma. The xylem vessels are fragmented in one or more transverse planes, according to the number of tiers of cells involved in abscission. Tyloses in various degrees of development and in various numbers are to be seen both above and below the abscission plane, but, as SWART[14] has shown experimentally, they cannot effectually hinder the

[14] SWART, N., Die Stoffwanderung in ablebenden Blaettern. Jena. 1914.

passage of water, since they are not sufficient either in size or numbers, and are not present at all in many vessels. The formation of tyloses appears to be rather a wound response, more or less incomplete at the time of rupture of the leaf, since the greater development, if incomplete, is found below the abscission plane, that is, the tyloses appear in the general region and at the same time as the general wound response.

The younger phloem elements, those quite near the cambium cells and with difficulty distinguishable from them, appear to behave the same as the latter. The older, on the other hand, which show a development of callus (though whether this is synchronous with the development of tyloses, as TISON holds, I cannot at present say) show indications that they behave in a quite passive manner. One can detect no definite zone of thinning in the wall, but, if stretched at all, they act merely as a soft yielding material, becoming thin throughout their whole length, quite as a soft india-rubber band behaves, and finally break. The very small size of the elements makes it difficult to be quite sure of this conclusion, but I have seen no evidence which would lead to any other.

The abscission cells of the epidermis behave as do the parenchyma cells, except for a certain asymmetry due to the unyielding cuticle, which merely breaks at last, after being loosened by alteration of the adjacent cellulose membrane.

The final condition of the active abscission cells is then as follows. The walls are locally, and it may be very irregularly, much extended and extremely thin. The thin membrane of each cell is distinct from that of any neighboring cell, and also from the proximal end walls of the cells above. The cytoplasm consists of a correspondingly delicate membrane, but shows no alteration in the direction of degeneration. The same is equally true of the nucleus, which is in appearance quite the same as evidently normal nuclei elsewhere, but that it is frequently much larger. When this condition has been reached, abscission may be regarded as complete. After the cuticle is broken, the abscission cells near by quickly collapse, while the shrinkage of the parts above, due to curtailment of water caused by local evaporation and by the severance of the vascular elements, produces a shearing and rupture of the abscission cells. At a slight touch, the whole layer of weakened

cell wall cells gives way, partly by pulling away the entire thinned walls (pl. figs. 5, 8) and partly by tearing them. The resulting wound surfaces of the separated parts seem, both to sight and touch, mucilaginous, as HANNIG observes, but this is not due to the entire dissolution of abscission cells, but to the fact that, on account of the thinness of the walls, many of them break, allowing their mucilaginous contents, accompanied by protoplasm, to ooze out on to the exposed wound surface. The contrast, in this respect, with the analogous surfaces of other plants (notably for example, in *Parthenium* and *Impatiens*) cannot be adduced as evidence of the nature of the process leading up to it. The only thing we might say of it is that it would lead one to examine the antecedent facts more closely, in order to detect essential differences, should they obtain. In this case I believe there are none.

Summary

1. Previous to abscission activity proper there is no antecedent structural indication of the position of the abscission zone. In young organs usually only one tier of cells is involved, while in old ones (internodes) evidence of physiological activity is to be seen in 10–12 tiers (the *abscission zone*) approximately. The greatest activity is to be seen in 1–5 tiers of cells, constituting the *separation layer*, at the upper limit of the abscission zone. Here new transverse walls occur in varying numbers, giving rise to the "Folgemeristem" of VON MOHL. The parenchyma at the base of a mature internode shows many transverse walls, which, however, have nothing to do with abscission, and do not constitute the criterion of an abscission zone. HANNIG'S "Lösungsschicht" and the "separation layer" of this paper may be regarded as identical, while his "Trennungsschicht" does not coincide with the "abscission zone" as here conceived.

2. Abscission begins in the internode near its base at two points which lie in a plane normal to that of the opposed leaves, and in the innermost part of the cortex. From these two points it is propagated outwardly toward the epidermis, and inwardly toward and into the pith. When more than one tier of cells is engaged, the changes which overtake them usually progress most rapidly in the uppermost (most distal) tier.

3. The walls of all the cells of the abscission zone, as defined in this paper, are altered chemically during abscission. The greatest degree of alteration takes place in the cells which are actually involved in separation (namely, those of the separation layer), in which the alteration, by hydrolysis, proceeds so far as to procure the complete digestion of a part of the primary and secondary walls, thus allowing a great extenuation of the tertiary walls, their separation from each other, and from the only partially altered primary and secondary walls of the next distal tier of cells.

4. The separation layer is composed of one tier of cells only in very young organs, and from 1 to 5 in older. Of these tiers, usually only the uppermost proceeds to complete separation. Mechanical resistances, which are greater in older parts, appear to influence the direction of the plane of separation.

5. The behavior of starch in the separation layer and adjoining tissues during abscission indicates that it is a source of energy for the separation cells during their growth.

6. The final separation results from the digestion of portions of the primary and secondary cell walls of the separation cells, leaving the protoplasts invested by a tertiary membrane which grows independently of adjoining membranes, from which it is quite free.

7. Neither the cytoplasm, nor the nuclei, nor any part thereof, displays degeneration changes. On the contrary, cytoplasm, nuclei, and nucleoli bear evidence of greater physiological activity, and are quite alive and normal when separation is achieved. There is meanwhile no loss of turgor.

Conclusion

From the foregoing facts it is concluded that abscission in *Mirabilis* is not procured by a separation resulting from the complete solution and destruction of a layer of tissue, as held by HANNIG, and does not therefore constitute a new type of abscission. Contrariwise, the mode of abscission accords wholly, as to all essential details, with that which has been shown to occur in such forms as *Gossypium*, *Aristolochia*, etc.

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EXPLANATION OF PLATE XIII

FIGS. 1–4.—Longitudinal sections through the bases of internodes, displaying variations in the regularity of form and number of separation layers; in fig. 1, a single tier of cells, in fig. 2, two tiers for a limited distance, in fig. 3, three tiers, and in fig. 4, four or even five tiers of cells are involved.

FIG. 5.—A small portion of the separation layer in the cortex in which abscission is complete; the abscission cells are more or less distorted, but those in the middle of the figure but little, and in these the normal nuclei can be seen; 3 cloudy masses can here be seen, and these are shown on a larger scale in fig. 6.

FIG. 6.—Longitudinal walls in a swelled and partly hydrolyzed condition; they can be seen to alternate with the protoplasts in position; ruthenium red; compare with figs. 11–13.

FIG. 7.—Abscission cells in the pith, when separation is readily possible; on the left of the central cell, the altered cell wall has broken, the tertiary membranes alone remaining intact; the protoplasm and nucleus are clearly normal.

FIG. 8.—The intact membranes of abscission cells remaining after separation has been procured; this preparation was secured by pulling apart a section such as that in fig. 5; at the free ends of the cells the membranes are seen to be somewhat thicker (cf. text fig. 1, *d*).

FIG. 9.—Cortical tissue in a very early stage of abscission, showing the reduced staining capacity of the walls of the cells proceeding toward abscission; Bismarck brown; there is no reduction in the thickness of these walls at this time.

FIG. 10.—A more advanced condition, in which the walls are thin and drawn out; there is further shown the transfer of the abscission plane from one cell tier to another; small, relatively unaltered portions of cell walls are seen suspended by the thin membranes.

FIG. 11.—Between the two protoplasts may be seen an oval intercellular space inclosed by two delicate tertiary membranes; these have been set free by the complete hydrolyzation of the remainder of the wall, of which nothing more is to be seen.

FIG. 12.—Medullary ray cells in abscission, in which the cell walls are hydrolyzed so far that they now appear granular or flocculated; only the wall in the center of the figure shows this well; this has been further magnified and is shown in fig. 13, in which can be seen the tertiary membrane on the right of the altered cell wall; ruthenium red.

FIG. 14.—The fragmentation of the wood vessels by the wood parenchyma and cambium cells which undergo active abscission; tyloses may be seen.

FIG. 15.—Medullary ray tissue, in which several tiers of cells are in a somewhat advanced stage of abscission; thicker walls show pits *en face;* the great delicacy of the thinned out membranes is well brought out here.